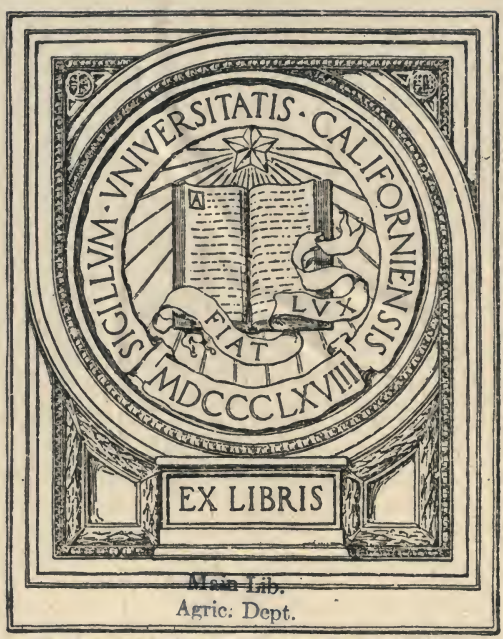

Issued July 30, 1910.

United States Department of Agriculture,

BUREAU OF CHEMISTRY—Circular No. 58.

H. W. WILEY, Chief of Bureau.

THE EFFECT OF ALCOHOL ON INVERTASE.

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PURPOSE OF THE INVESTIGATION.

A knowledge of the action of alcohol on invertase is of practical importance for two reasons; first, alcohol is naturally present during the fermentation of cane sugar by yeast and the invertase of the yeast is thus normally in the presence of weak alcohol; and, second, alcohol is often used, though generally with little success, to prepare the enzym in a solid form by precipitation from an aqueous extract of yeast. In order to learn what influence alcohol of different strengths has on invertase the following investigation was made. The results show that the influence is exceedingly great and that it consists in three distinct actions, namely, an inactivation, a permanent destruction, and a precipitation of the enzym. These actions will be described in the order named.

THE INACTIVATION OF INVERTASE BY ALCOHOL.

The activity of purified invertase in inverting cane sugar dissolved in various strengths of ethyl alcohol was measured at 30° C. by the usual method. Care was taken to have sufficient acetic acid in the solutions to insure that the maximum activity of the enzym was attained, and the solutions were made alkaline at the end of the experiment to stop the enzymotic action and complete the mutarotation. The alcohol used was Squibb's or Baker's absolute alcohol and the concentrations were expressed as volume per cent. The

activities are recorded in Table 1 as the velocity-coefficients of the inversion, using minutes and decimal logarithms and multiplying by 10,000 to avoid decimals. The results are also expressed graphically in figure 1.

It is apparent that alcohol has a strong inactivating action on invertase even when the alcohol is dilute. The destruction of invertase by alcohol does not take place below about 20 per cent, as will be shown under the following caption, but the activity of the enzym is markedly lowered by even a few per cent of alcohol. The

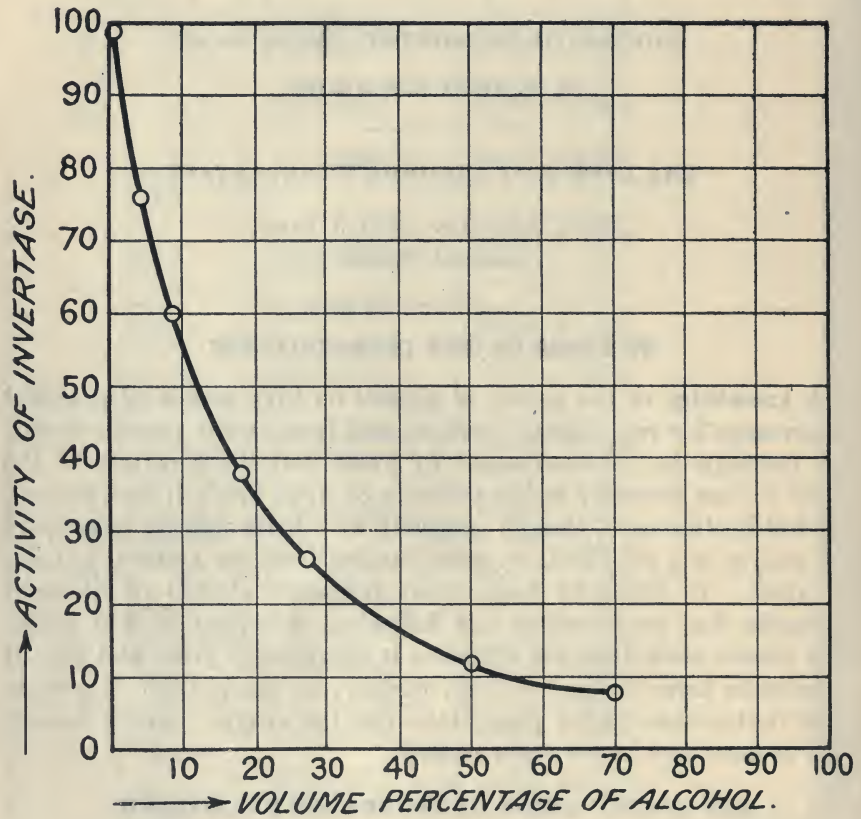

FIG. 1.—The influence of alcohol on the activity of invertase.

graph shows that the relation between alcoholic strength and activity is not proportionality but may be represented as a logarithmic or hyperbolic function. On account of the fact that rapid destruction of the enzym takes place in solutions above 30 per cent alcoholic strength it is not possible to measure the activity of invertase in this region by the usual method; the theory of the modified procedure which has accordingly been used to measure the activity in 50 and 70 per cent alcohol is given on page 6 and the values which were found are included in the table and figure.

TABLE 1.—*Activity of purified invertase in aqueous alcohol.*

[Temperature 30° C. Concentrations: Aqueous alcohol containing cane sugar 0.2 normal, and acetic acid 0.02 normal; 100 cc of this mixture were added to 10 cc of purified invertase solution.]

Concentration of alcohol (volume per cent.)	Activity of invertase k_1 (10,000).	Percentage activity.
0	80	100
4.3	61	76
9.1	48	60
18.2	30	38
27.3	21	26
50.0	8.8	11
70.0	6.4	8

The inactivation of invertase by alcohol was noticed by O'Sullivan and Tompson,[a] and they state that the inactivation by alcohol is "in direct proportion with the amount present. Five per cent alcohol decreases the speed of the reaction by about one-half." The results here given are somewhat different in detail from those quoted, because we have measured the inactivation for higher strengths of alcohol than were used by the previous investigators. It will be noticed that our results also show almost a linear relation between activity and alcohol strength for weak alcoholic solutions.

THE DESTRUCTION OF INVERTASE BY ALCOHOL.

The measurements of the destruction of invertase by alcohol were made by the method previously used in studying the destruction of the enzym by acids and alkalis.[b] The rate of the destruction follows the course of unimolecular reactions, as is shown by the following experiment. The value of the velocity-coefficient of the unimolecular formula, k_2, remains constant within the errors of experiment during the course of the destruction.

TABLE 2.—*Unimolecular order of the destruction of invertase by alcohol.*

[Temperature 30° C. Alcohol 20 per cent by volume. Maximum activities were measured.]

Time.	Activity of invertase [k_1 (10,000)].	Velocity coefficient (k_2).
Minutes.		
0	8.98	.....
15	8.11	0.00295
30	7.33	.00294
45	6.54	.00306
75	5.41	.00293
105	4.66	.00271
165	4.13	.00204
Average..	.....	.00277

[a] J. Chem. Soc., 1890, 57:927.
[b] U. S. Dept. Agr., Bureau of Chemistry Cir. 55.

The rates of destruction of invertase which were found in various strengths of alcohol are recorded in Table 3, the values of k_2 being expressed in minutes and decimal logarithms and multiplied by 1,000 to avoid decimals. The results are also shown graphically in figure 2.

TABLE 3.—*Rates of destruction of invertase by alcohol.*

Concentration of alcohol (volume per cent).	Rate of destruction [$k_2 \times (1{,}000)$].	Concentration of alcohol (volume per cent).	Rate of destruction [$k_2 \times (1{,}000)$].
0	0	50	850
10	0	55	570
20	3	60	240
30	44	70	74
40	260	80	7
45	487	90	2

The rate of destruction changes most peculiarly with the strength of the alcohol, reaching a pronounced maximum at about 50 per cent. Presumably the protective action of strong alcohol is due to its precipitating the invertase, or some other substance whose precipitation protects the invertase, as a visible precipitation begins at or near the strength of 50 per cent, at which the maximum of the curve falls and the protective action begins. If the curve to the left of the maximum is alone regarded as the curve of the destruction in solution it is noticed that it is very similar to the curve for the destruction of the enzym by acids and alkalis.[a] It appears, therefore, that the destruction by alcohol in homogeneous solution is due to a decomposition of the invertase similar to the hydrolytic decompositions that are presumably the cause of the destruction by acids and alkalis. The practical application of the results on alcoholic precipitation is given on page 7.

THE ACTION OF CANE SUGAR IN PREVENTING THE DESTRUCTION OF INVERTASE.

In order to learn whether cane sugar protects invertase from destruction by alcohol the rate of destruction in a 0.2 normal solution of cane sugar in 50 per cent alcohol was measured at 30° C. by the method already described. A correction was applied for the rotation of the sugar in the samples as they were removed to test their activities. The method of preparing the mixture of cane sugar, aqueous alcohol, invertase, and acetic acid is shown in the description of the experiment recorded in Table 5; the results on the rate of destruction are given in Table 4.

[a] U. S. Dept. Agr., Bureau of Chemistry Cir. 55, p. 6.

TABLE 4.—*Rate of destruction of invertase in 50 per cent alcohol containing 6 per cent of cane sugar.*

Time.	Activity of sample (k_1).	Velocity coefficient (k_2).
Minutes.		
0	0.00049	.....
30	.00024	0.010
60	.00016	.008
Average..	.....	.009

This rate of destruction is far less than that shown for 50 per cent alcohol in Table 3, namely, 0.850. The cause of this difference can lie only in the fact that cane sugar was not present in the latter

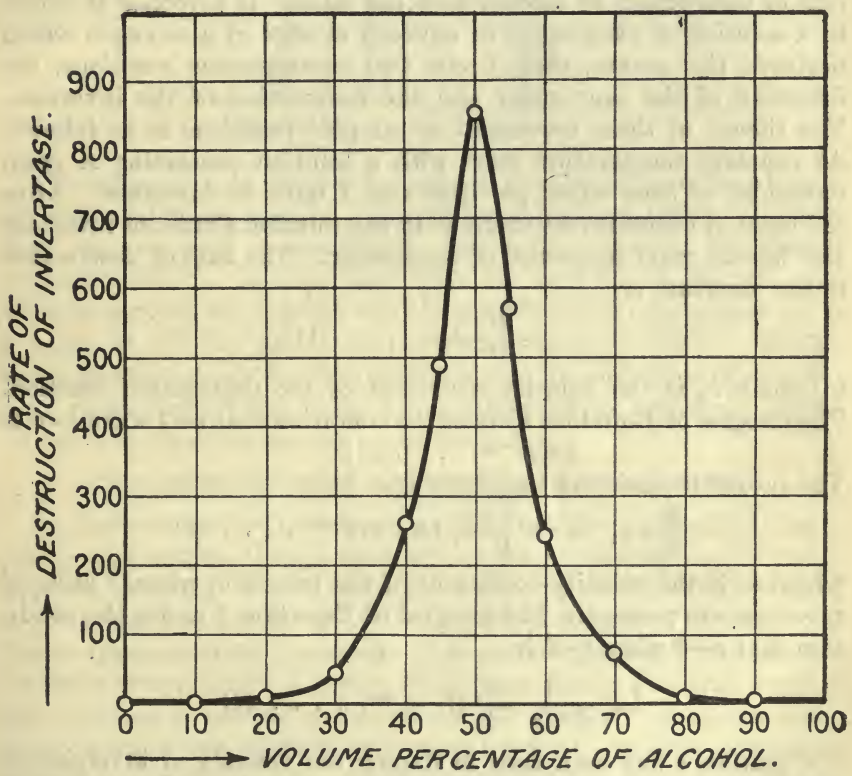

FIG. 2.—The destruction of invertase by alcohol.

experiments. Several other experiments have given results agreeing with these in showing that cane sugar exerts a very strong influence in protecting invertase from destruction by alcohol. O'Sullivan

and Tompson [a] have shown that cane sugar protects invertase from destruction by hot water, and it is well known that diastase is not so easily destroyed by hot water if maltose is in the solution; it is then an analogous fact that cane sugar protects invertase from the action of alcohol. The most plausible explanation is that these sugars combine with the enzym, and the resulting compound is not so easily attacked by alcohol or hot water. In Circular No. 59 further data are presented on this interesting subject.

THEORY OF THE RATE OF INVERSION OF CANE SUGAR DURING THE DESTRUCTION OF THE INVERTASE.

The rate of inversion of cane sugar by invertase in dilute solution follows the unimolecular order and it has just been shown that the rate of destruction by alcohol does the same. If invertase is added to a solution of cane sugar in aqueous alcohol of a strength which destroys the enzym, there occur two simultaneous reactions, the inversion of the cane sugar and the destruction of the invertase. The theory of these dependent or coupled reactions is as follows: At constant temperature start with a solution containing A gram molecules of cane sugar per liter and I units of invertase. After the lapse of t minutes let there be in the solution i units of invertase and $(A-x)$ gram molecules of cane sugar. The rate of destruction of the invertase is

$$-\frac{di}{dt}=k_2 i \quad . \quad . \quad . \quad . \quad (1)$$

in which k_2 is the velocity-coefficient of the destruction reaction. The integral of Equation 1 under the condition that $i=I$ when $t=0$ is

$$i=Ie^{-k_2 t} \quad . \quad . \quad . \quad . \quad (2)$$

The rate of inversion of the cane sugar is

$$\frac{dx}{dt}=k_1 (A-x)\frac{i}{I}=k_1 (A-x)\, e^{-k_2 t} \quad . \quad . \quad . \quad . \quad (3)$$

where k_1 is the velocity-coefficient of the inversion when I units of invertase are present. The integral of Equation 3 under the condition that $x=0$ when $t=0$ is

$$\log \frac{A}{A-x}=\frac{k_1}{k_2}(1-e^{-k_2 t}) \quad . \quad . \quad . \quad . \quad (4)$$

Equation 4 has been used in finding the velocity of inversion of cane sugar by invertase (k_1) in 50 per cent alcohol. A mixture of 600 cc of 0.2 normal cane sugar solution in 55 per cent alcohol (which was also 0.02 normal with respect to acetic acid) with 60 cc of dialized invertase solution was kept at 30° C. and the progress of the inversion measured polarimetrically from time to time, the samples being made alkaline before each reading of the rotation. In Table 5

[a] J. Chem. Soc., 1890, **57**:900.

the rate of this incomplete inversion of cane sugar by invertase in 50 per cent alcohol is recorded. The quantity of cane sugar present (A) is expressed in degrees, $A=48.1\ (1.267)^a=61.6$, and the quantity of cane sugar present at any time t is $A-x=r+48.7\ (0.267)$, where r is the reading of the solution. The value of k_2 is taken from Table 4 as 0.009.

TABLE 5.—*Course of the incomplete inversion of cane sugar by invertase in 50 per cent alcohol.*

Time.	Rotation (r).	$k_1=\frac{k_2}{1-10^{-k_2 t}}\log\frac{A}{A-x}$.
Minutes.		
0	48.7	.....
15	44.8	0.00094
30	42.3	.00091
45	40.9	.00087
60	39.9	.00084
90	38.5	.00083
120	37.7	.00083
240	36.0	.00086
360	35.6	.00094
Average..	.....	.00088

The values of k_1 in the last column, as calculated from Equation 4, are sufficiently constant to show that the reaction follows the laws that were assumed in the theory, within the limits of the present experimentation. It was found that the activity of the invertase when no alcohol was present was $k_1=0.0080$; if this rate in pure water is taken as 100, the rate in 50 per cent alcohol is then 11. Similar experiments have shown that in 70 per cent alcohol the rate of inversion, on the basis of 100, is 8. These values for the activity in 50 and 70 per cent alcohol are included in Table 1.

THE PRECIPITATION OF INVERTASE BY ALCOHOL.

Alcohol precipitates invertase, and it is in this way possible to prepare a solid enzym, though nothing regarding its purity can be predicated at present. It is a fact, however, that such solid invertase preparations are usually of low enzymotic activity. The reason for this is apparent from the results of this investigation, for unless the alcoholic precipitation is performed in very strong alcoholic solution the invertase is rapidly destroyed by the alcohol. There are three ways by which this destruction can be lessened; one is to use strong alcohol in large proportion, another is to work at low temperatures, and the third is to have cane sugar present in the solution to protect the invertase from destruction. In order to test the first method, 50 cc of invertase liquor which had been dialyzed until it contained only 1 per cent of total solids was mixed with 500 cc of 95 per cent alcohol at 25° C., and after half an hour the coagulated precipitate was

[a] The Clerget factor at 30° C; see U. S. Dept. Agr., Bureau of Chemistry Cir. 50, p. 3

filtered off, washed with alcohol, then with ether, and dried over sulphuric acid in a desiccator. The mass, which resembled horn in superficial appearance, was dissolved in 50 cc of water, and its inverting activity was found to be 78 per cent of that of the original sample. The second method of precipitation, at low temperature (0° C.), gave a small recovery and is not to be recommended.

In order to test the protective action of cane sugar, 25 grams of it were dissolved in 50 cc of dialyzed invertase and 95 per cent alcohol added until the strength of the mixture became 70 per cent alcohol. The precipitate was filtered off, washed with ether, and dried in a desiccator. On redissolving, it showed 94 per cent of the original activity and a second experiment gave a recovery of 96 per cent. These experiments demonstrate that it is possible to precipitate invertase with alcohol without any important loss of activity provided the enzym is protected by cane sugar. Other sugars may have this protective action also, but this point has not yet been investigated.

SUMMARY.

O'Sullivan and Tompson's observation that alcohol reduces the activity of invertase is confirmed, and the relation between alcoholic strength and inactivation is shown to be graphically a rounded curve (fig. 1). Alcohol is found to destroy invertase, and the relation between alcoholic strength and rate of destruction is very peculiar, as it shows a high maximum at about 50 per cent alcohol. The destruction follows the course of unimolecular reactions; it is not noticeable below 20 per cent alcohol at 30° C., is almost instantaneous at 50 per cent, and decreases to nearly zero at 80 per cent (fig. 2). If the alcohol contains cane sugar, the destruction is much slower; thus, 6 per cent cane sugar reduces the rate of destruction in 50 per cent alcohol from 0.850 to 0.009, or to about 1 per cent of its original value. A mathematical theory of the progress of the inversion of cane sugar by invertase in alcoholic solutions of sufficient strength to slowly destroy the enzym has been worked out and its conclusions found to agree with the results of the experiments. In this way it has been possible to measure the activity of invertase in 50 and 70 per cent alcohol, where the destruction plays an important rôle. Invertase can be precipitated by alcohol without much destruction, provided the strength of alcohol in the final solution is high, approximately 90 per cent. By this method of precipitation, working at room temperature, a solid preparation was obtained which had 78 per cent of the activity of the original solution. If cane sugar is present, invertase can be precipitated with no important destruction by even 70 per cent alcohol; this method of precipitation gave a recovery of 94 and 96 per cent of the original activity.

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